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(54) Title: CYCLIC CS-1 PEPTIDOMIMETICS, COMPOSITIONS AND METHODS OF USING THE SAME

(57) Abstract

The present invention contemplates a cyclic peptide that inhibits the binding between the VLA-4 receptor expressed on inflammatory leukocytes and the fibronectin CS-1 peptide expressed on endothelial cells that are involved in immunoinflammatory disease states. Pharmaceutical compositions containing a contemplated cyclic peptide and processes for treating immunoinflammatory conditions using a binding-inhibitory cyclic peptide are also disclosed.

CYCLIC CS-1 PEPTIDOMIMETICS, COMPOSITIONS AND METHODS OF USING THE SAME

Description

Technical Field

The present invention relates to binding of inflammatory cells to endothelial cells that express the CS-1 portion of fibronectin on their surfaces, and more particularly to the inhibition of that binding by cyclic peptidomimetic compounds of minimal length.

Background Art

The immune response relies on leukocyte trafficking and immune surveillance as one of the underpinnings of host defense. Not only does this immune surveillance allow leukocytes to recirculate through lymphoid tissues normally, but also permits rapid leukocyte recruitment and extravasation to adjacent tissues at sites of inflammation. The $\alpha 4\beta 1$ (CD49d/CD29, VLA-4) cell adhesion receptor is an active participant in these leukocyte trafficking functions [Hemler, Ann. Rev. Immunol., 8:365-400 (1990); Hemler et al., Immunol. Rev., 114:45-65 (1990)].

The VLA-4 integrin heterodimer was discovered independently by three research groups and identified as a surface antigen on lymphocytes [Sanchez-Madrid et al., Eur. J. Immunol., 16:1343-1349 (1986); Clayberger et al., J. Immunol., 138:1510-1514 (1987); Hemler et al., J. Biol. Chem., 262:11478-11485 (1987)]. Within the integrin family, VLA-4 is unique on several counts: (i) in contrast to

two separate counterreceptor structur s, namely, the cytokine-inducible vascular cell adhesion molecule-1 (VCAM-1) [Elices et al., Cell, 60:577-584 (1990); Rice et al., J. Exp. Med., 171:1369-1374 (1990); Schwartz et al., J. Clin. Invest., 85:2019-2022 (1990); Carlos et al., Blood, 76:965-970 (1990)], and a subset of the ubiquitous ECM protein fibronectin [Wayner et al., J. Cell Biol., 109:1321-1330 (1989); Guan et al., Cell, 60:53-61 (1990); Ferreira et al., J. Exp. Med., 171:351-356 (1990); Elices et al., Cell, 60:577-584 (1990)].

VCAM-1 is a member of the immunoglobulin (Ig) gene superfamily [Osborn et al., Cell, 59:1203-1211 (1989); Rice et al., Science, 246:1303-1306 (1989)] that is expressed predominantly in vascular endothelium in response to pro-inflammatory cytokines such as IL-1, TNFα, and IL-4 [Osborn et al., Cell, 59:1203-1211 (1989); Rice et al., Science, 246:1303-1306 (1989); Thornhill et al., J. Immunol., 145:865-872 (1990); Masinovsky et al., J. Immunol., 145:2886-2895 (1990); Thornhill et al., J. Immunol., 146:592-598 (1991); Schleimer et al., J. Immunol., 148:1086-1092 (1992); Birdsall et al., J. Immunol., 148:2717-2723 (1992); Swerlick et al., J. Immunol., 149:798-705 (1992); Briscoe et al., J. Immunol., 149:2954-2960 (1992)]. The VLA-4 binding sites on VCAM-1 have been mapped to the outermost N-terminal (first) Ig-like region of the 6-Ig-like domain VCAM-1 isoform [Taichman et al., Cell Regul., 2:347-355 (1991); Vonderheide et al., J. Exp. Med., 175:1433-1442 (1992); Osborn et al., J. Exp. Med., 176:99-107 (1992)], and the first and fourth N-terminal Ig-lik regions of the 7-Ig-like domain VCAM-1 isoform [Vonderheide et al., J. Exp. Med., 175:1433-1442 (1992); Osborn et al., J. Exp. Med., 176:99-107 (1992)]. Discrete amino acid s quences within the

published August 20, 1992] albeit VLA-4 binds to LDV with at least two orders of magnitude lower affinity than to the native CS-1 25-mer.

Nowlin et al., J. Biol. Chem., 268(11):20352-20359 (1993) recently described a cystine-linked cyclic pentapeptide said to inhibit binding by both the Arg-Gly-Asp (RGD) and CS-1 regions of fibronectin to VLA-5 and VLA-4, respectively. That cyclic pentamer included the unnatural residue, thioproline (ThioP), and can be represented by Arg-Cys*-Asp-(ThioP)-Cys*, that is cyclized through a disulfide bond formed at the starred cysteine residues.

VLA-4 shares with other members of the β1 integrin subfamily the ability to promote binding and penetration of microbial pathogens into mammalian cells. Thus, specific interactions of β1 integrins with the bacterial protein invasin [Isberg et al., Cell, 60:861-871 (1990); Ennis et al., J. Exp. Med., 177:207-212 (1993)], as well as the protozoan Trypanosoma cruzi [Fernandez et al., Eur. J. Immunol., 23:552-557 (1993)] have been described.

A multitude of in vitro studies suggest interactions of VLA-4 with its two known ligands, VCAM-1 and CS-1 FN, have profound biological significance. For instance, VLA-4 binding to VCAM-1 has been demonstrated in adhesion to cytokine-stimulated vascular endothelium by lymphocytes [Elices et al., Cell, 60:577-584 (1990); Rice et al., J. Exp. Med., 171:1369-1374 (1990); Schwartz et al., J. Clin. Invest., 85:2019-2022 (1990); Carlos et al., Blood, 76:965-970 (1990); Shimizu et al., J. Cell Biol., 113:1203-1212 (1991)], monocytes [Carlos et al., Blood, 77:2266-2271 (1991); Jonjic et al., J. Immunol., 148:2080-2083 (1992)], natural killer (NK) cells [Allavena et al., J. Exp. Med., 173:439-448 (1991)], and eosinophils [Walsh et al., J. Immunol.,

88:546-552 (1992)]. A role for the CS-1 splicing variant of FN has been established in mediating migration of inflammatory cells such as eosinophils across endothelial cell monolayers of VLA-4-expressing leukocytes [Kuijpers et al., J. Exp. Med., 178:279-284 (1993)]. Recent studies have also documented that the expression of the CS-1 variant of FN is increased in human patients whose bodies reject transplanted kidneys.

The vast body of work suggesting that VLA-4 plays a role in leukocyte trafficking and inflammation has been largely confirmed by in vivo studies using anti-VLA-4 antibodies in various animal models. Essentially, the skin, brain, kidney, lung and gut are targets of a wide variety of VLA-4-dependent inflammatory reactions mostly resulting from recruitment of mononuclear leukocytes and eosinophils.

More specifically, these in vivo studies are as follows: contact hypersensitivity (CH) and delayed type hypersensitivity (DTH) in the mouse and rat [Ferguson et al., Proc. Natl. Acad. Sci. USA, 88:8072-8076 (1991); Issekutz, Cell Immunol., 138:300-312 (1991); Issekutz, J. Immunol., 147:4178-4184 (1991); Elices et al., Clin. Exp. Rheumatol., 11:S77-80 (1993); Ferguson et al., Proc. Natl. Acad. Sci. USA, 88:8072-8076 (1991); Chisholm, et al., Eur. J. Immunol., 23:682-688 (1993)]; experimental autoimmune encephalomyelitis (EAE) in the mouse and rat [Yednock et al., Nature, 356:63-66 (1992); Baron et al., J. Exp. Med., 177:57-68 (1993)]; nephrotoxic nephritis in the rat [Mulligan et al., J. Clin. Invest., 91:577-587 (1993)]; passiv cutaneous anaphylaxis in the guinea pig [Weg et al., J. Exp. Med., 177:561-566 (1993)]; immune complex-induced lung injury in the rat [Mulligan et al., J. Immunol., 150:2401-2406 (1993); Mulligan et al., J. Immunol.,

The role of VLA-4 and the CS-1 peptide in various chronic and acute immunoinflammatory disease states having been established, it would be of importance if compounds could be found that inhibit the VLA-4-lymphocyte interaction and were other than anti-VLA-4 antibodies that can themselves induce an immune response on repeated administration or the CS-1 peptide that is large and costly to make, and also is subject to rapid degradation. The disclosure that follows describes such small molecules that are more potent than is CS-1 itself.

Brief Summary of the Invention

The present invention contemplates a cyclic CS-1 peptidomimetic inhibitor peptide, compositions and methods (processes) for using such an inhibitor peptide.

A contemplated cyclic peptide corresponds in sequence to formula I of SEQ ID NO:4-28

$$R\text{-}Xaa^1\text{-}Z\text{-}Asp\text{-}Phe\text{-}Y\text{-}Xaa^2 \qquad I$$

wherein

R is

(a) R^1 that is (i) absent so that the peptide is terminated by the free α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or

(b) R^2 that is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tyrosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl;

the Xaa^1 and Xaa^2 groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which Xaa^2 has a C-terminal carboxamide group, or the C-terminal

(a) R^1 that is (i) absent so that th peptide is terminated by th free α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or

(b) R^2 that is selected from the group consisting of phenylacetyl, phenylalanyl and N-C_1-C_4 acyl phenylalanyl;

at least one Xaa is an oxidized cysteine and the other Xaa is an oxidized cysteine, homocysteine or penicillamine residue such that the two Xaa's together form a disulfide bond;

Z is absent, or a peptide selected from the group consisting of Pro-Glu-Leu, Phe-Leu, Glu-Phe-Leu, Pro-Glu-Phe-Leu (SEQ ID NO:29), and Gly-Pro-Glu-Phe-Leu (SEQ ID NO:30); and

Y is absent, Pro, or Y^1 that is a peptide selected from the group consisting of Pro-Ser, Pro-Ser-Thr and Pro-Ser-Thr-Val (SEQ ID NO:31);

with the provisos that:

(i) R is R^2 when Z is absent, and

(ii) R^2 is phenylacetyl or N-C_1-C_4 acyl phenylalanyl when the two Xaa residues are separated by two amino acid residues; i.e., when both Z and Y are absent.

In a still more preferred embodiment, the cyclic peptide corresponds in sequence to formula II, below,

R-Xaa-Z^1-Asp-Phe-Y^2-Xaa-NH_2, (SEQ ID NO:5-7, 9-12) II

wherein

R is

(a) R^1 that is (i) absent so that the peptide is terminated by th fr e α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or

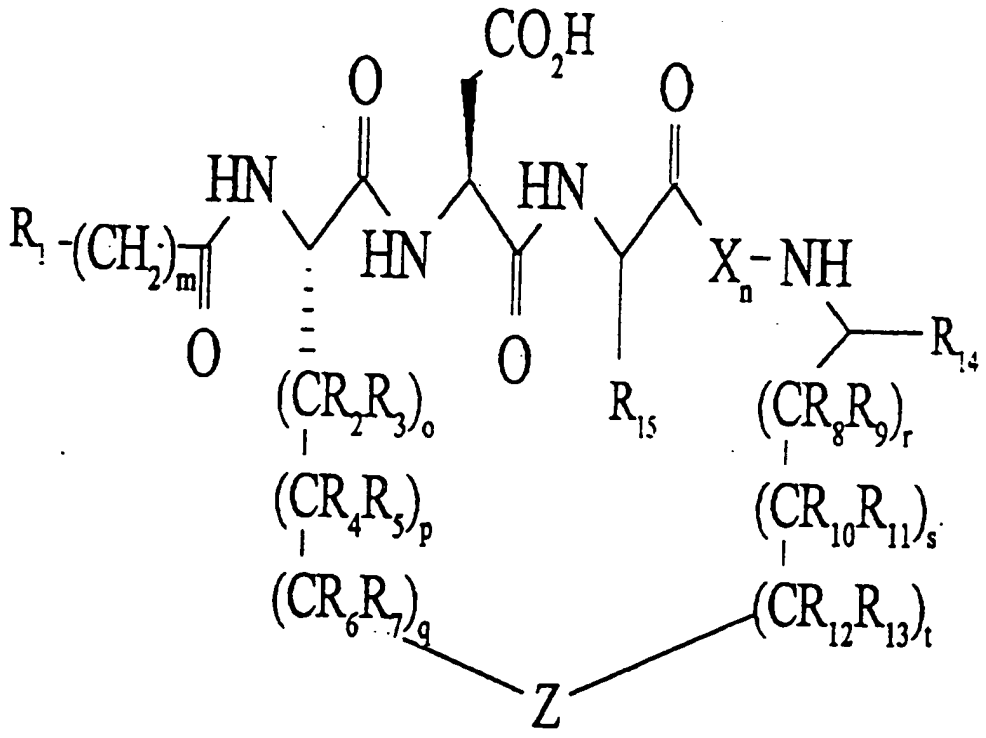
(b) R^2 that is selected from the group consisting of phenylacetyl, phenylalanyl and N-C_1-C_4 acyl phenylalanyl;

herein as a CS-1/ or sVCAM-1/VLA-4-inhibiting amount. An above-discussed mor preferred, still more preferred or most preferred peptide is utilized in a more preferred, still more preferred or most preferred composition.

A process for treating fibronectin CS-1/ or sVCAM-1/VLA-4-mediated inflammation is also contemplated. That process comprises administering to a mammal having that inflammation or otherwise in need of such a treatment as for prophylactic purposes, an inflammation-reducing amount of a before-described cyclic inhibitor peptide. Use of a more preferred, still more preferred or most preferred inhibitor peptide is more, still more or most preferred in this process. The cyclic inhibitor peptide is preferably administered in a before-described pharmaceutical composition.

All peptide formulas or sequences shown herein are written from left to right and in the direction from amino-terminus to carboxy-terminus. The abbreviations used herein for derivatives and residues of the twenty natural amino acids are reproduced in the following Table of Correspondence:

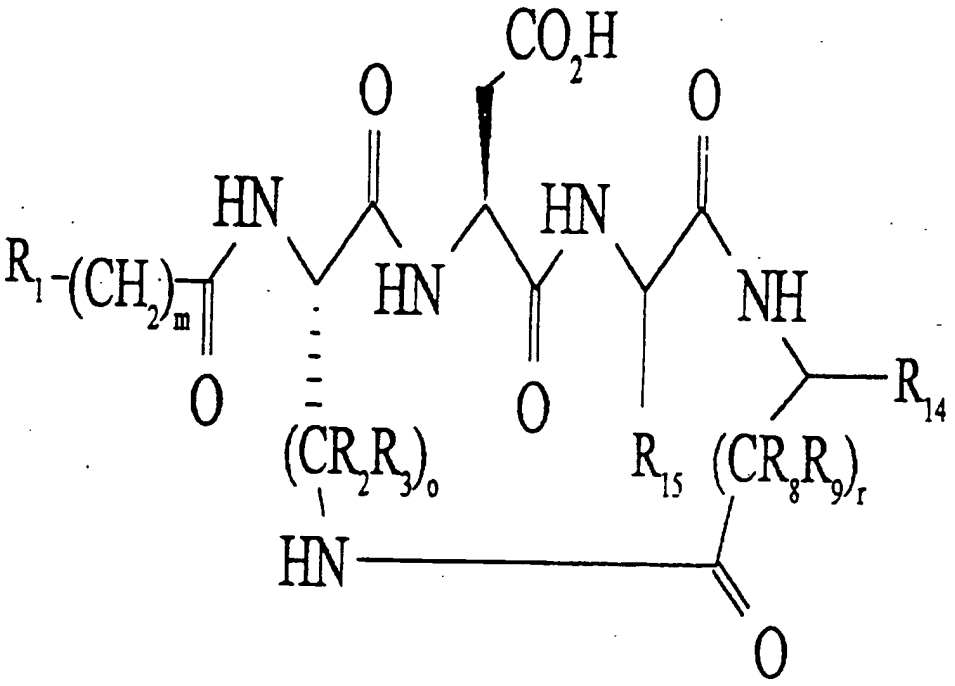
Alternatively, th cyclic inhibitor peptide of the present invention can be described in molecular formula form. In this form, a preferred cyclic inhibitor peptide has the following formula:



Z is a linker group selected from the group consisting of -S-S-, -NH-C(O)-, -S-, and -C(O)-NH-. X_n is 0 to about 6 amino acids and subscript m is 0 to 5. R_1 is a phenyl, pyridyl or thiophenyl group optionally substituted by one or more lower alkyl, C_1 to C_3 alkoxy, halo, amino, C_1 to C_3 acylamino or nitro groups. The subscripts o, p, q, r, s, and t are, independently, 0 or 1 and R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, R_{11}, R_{12}, and R_{13} are, independently, a hydrogen atom or a lower alkyl or phenyl group. R_{14} is a hydrogen atom or a primary amide, carboxylic acid, lower alkyl ester, thiolo ester or mono or dihydroxy lower alkyl group. R_{15} is a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group or a

the lower alkyl group is a C_1 to C_5 alkyl straight or branch chain moiety or a salt ther of.

Further, a preferred cyclic inhibitor peptide can be described by the following formula:



Where subscript m is 0 to 5 and R_1 is a phenyl, pyridyl or thiophenyl group optionally substituted by one or more lower alkyl, C_1 to C_5 alkoxy, halo, amino, C_1 to C_5 acylamino or nitro groups. Subscript o and r are, independently, 0 or 1 and R_2, R_3, R_8, and R_9 are, independently, a hydrogen atom or a lower alkyl group. R_{14} is a hydrogen atom or a primary amide, carboxylic acid, lower alkyl ester, thiolo ester or mono or dihydroxy lower alkyl group. R_{15} is a ph nyl, pyridyl, cyclohexyl, thiophenyl or primary amide group or a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group wherein

Further, prodrug forms and pharmaceutical compositions of the above formulas are also encompassed by the present invention.

The present invention has several benefits and advantages.

One salient benefit is that a cyclic inhibitor peptide contemplated here is more potent in inhibiting the VLA-4/CS-1 or the VLA-4/sVCAM-1 binding interaction than is CS-1 itself.

An advantage of the invention is that a contemplated cyclic inhibitor peptide also inhibits binding between VLA-4 and VCAM-1 to a greater extent than does a straight chain peptide that exhibits similar binding inhibition between VLA-4 and a CS-1 peptide.

Another advantage of the invention is that a contemplated cyclic inhibitor peptide is a relatively small molecule that is easily prepared in high purity.

Still further benefits and advantages of the invention will become apparent to the skilled worker from the disclosure that follows.

Brief Description of the Drawings

In the drawings forming a portion of this disclosure:

Fig. 1 is a graph illustrating the relative in vitro inhibition of binding of VLA-4-bearing Jurkat cells to the solid phase-bound CS-1 peptide (SEQ ID NO:1) on the left or soluble (s) sVCAM-1 on the right caused by straight chain peptides ϕAc-LeuAspPhe-morpholinamide (XLDFZ), ϕAc-LeuAspPhe-D-Pro-NH_2 (XLDFp) or the disulfid -containing cyclic p ptide CysAspPheCys-NH_2 (cyclic; SEQ ID NO:4), respectively. Inhibitions on the left are relative to the inhibition provided by the "standard" peptide of SEQ ID NO:3, GlyProGluIleLeuAspValProSerThr, whos

contemplated cyclic peptide will usually be discussed in regard to its CS-1-mimetic properties, but that is for the sake of convenience only. As will be seen from the discussion that follows, a contemplated peptide binds to the VLA-4 receptor even more tightly than does the CS-1 25-mer peptide present in fibronectin or the soluble form of VCAM-1, sVCAM-1.

Broadly, a contemplated cyclic inhibitor peptide can be defined as having a structure corresponding to formula I, below, SEQ ID NO:4-28

$$R\text{-}Xaa^1\text{-}Z\text{-}Asp\text{-}Phe\text{-}Y\text{-}Xaa^2 \qquad I$$

wherein

R is

(a) R^1 that is (i) absent so that the peptide is terminated by the free α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or

(b) R^2 that is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tyrosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl;

the Xaa^1 and Xaa^2 groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which Xaa^2 has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen;

Z is absent, or a peptide selected from the group consisting of Pro-Glu-Leu, Phe-Leu, Glu-Phe-Leu, Pro-Glu-Phe-Leu (SEQ ID NO:29), and Gly-Pro-Glu-Phe-Leu (SEQ ID NO:30); and

Y is absent, Pro, or Y^1 that is a peptide selected from the group consisting of Pro-Ser, Pro-Ser-Thr and Pro-Ser-Thr-Val (SEQ ID NO:31);

with the provisos that:

(i) R is R^2 when Z is absent, and

A cyclic peptide of formula I or Ia, as w ll as those of formulas II and III below, is water-soluble and inhibits the binding of Jurkat cells (ATCC TIB 152) to a solid phase-bound peptide of SEQ ID NO:1 in an in vitro assay in an aqueous buffer at a pH value of 7.2-7.4 to an extent that is about 5- to about 600-fold better (more potent) than the inhibition in said binding exhibited by a peptide of SEQ ID NO:3.

Examining formulas I and Ia, it is seen that only the Asp of the CS-1 (SEQ ID NO:1) and B12 (SEQ ID NO:2) fibronectin peptides is required to be present, as is a Phe residue that is not present in either CS-1 or B12. In addition to that two-residue sequence, the sequence/structure of a contemplated inhibitor peptide and the CS-1 or B12 portions can be similar, although a contemplated peptide here is cyclic, whereas the CS-1 and B12 peptides are linear. Still further, a most preferred cyclic inhibitor peptide is quite different in sequence from a CS-1 or B12 peptide.

In examining formulas I and Ia it is also seen that the N-terminal R group can be an R^1 group that is absent so that the N-terminus of a cyclic peptide is the free α-amine of the Xaa^1 residue. R can also be an R^1 group that is a C_1-C_4 acyl moiety or a salt thereof. Exemplary C_1-C_4 acyl moieties include formyl, acetyl (which is preferred), propionyl, n-butanoyl and iso-butanoyl.

As already noted, an R group can also be an R^2 group. An R^2 group is selected from the group consisting of phenylacetyl, phenylalanyl and N-C_1-C_4 acyl phenylalanyl.

As is noted in the above provisos, R is R^2 when Z is absent. In addition, when there are only two residues between the two Xaa residues of a cyclic

S r-Thr and Pro-Ser-Thr-Val (SEQ ID NO:31) and is sometimes r f rred to her in as Y^1.

Thus, the Asp-Phe-Y- portion of a cyclic inhibitor peptide can be viewed as both a substitution analogue of a CS-1 and B12 peptide because of the substitution of Val with Phe, and a deletion analog of those peptides because of the relative shortness of a contemplated peptide compared to peptide CS-1 or B12. Valine (Val) is even of smaller size than Ile, so that successful substitution of the much larger Phe side chain for the smaller Val side chain was again unexpected.

Formulas I and Ia contain two Xaa residues or Xaa^1 and Xaa^2 groups that are α-amino acid residues that together form a sulfide or disulfide bond in a chain (bridge) that contains 3 to about 6 atoms, including at least one sulfur atom, between the α-carbons of the two Xaa groups. The group Xaa^2 has C-terminal carboxamide [$-C(O)NH_2$)] group or the C-terminal carboxyl is replaced by hydrogen.

Thus, the two Xaa groups each contain an α-carbon atom and there is a sulfur atom-containing chain or bridge of atoms between those α-carbon atoms. The sulfur-containing chain can contain one sulfur atom as where a sulfide bond is present in lanthionine, or can contain two sulfur atoms of a disulfide bond as in cystine.

Chains of varying lengths that contain a disulfide bond can be formed by the oxidation of mercaptan-containing residues such as cysteine, homocysteine or penicillamine. Chains of varying length that contain a sulfide bond can be formed by reaction of one of the above mercaptan-containing residues, with an appropriately blocked β-, γ- or K-leaving group-containing amino acid such as 2-N-t-Boc-amino-3-O-methanesulfonylpropionic acid, 2-N-succinimido-4-bromobutyric acid or 2-N-succinimido-5-

code). More preferably that binding is inhibited by about 50- to about 600-fold, and most preferably by about 100- to about 600-fold better than that exhibited by the standard 10-mer.

Binding inhibition is measured here as a concentration of peptide that inhibits one-half the binding between a standard number of Jurkat cells and a standard amount of CS-1 peptide bound to the surface of a microtiter plate well. Those concentrations are conveniently expressed as IC_{50} values, smaller numbers indicating a lower concentration required to inhibit 50 percent binding and therefore greater potency. Further specifics of this assay are provided hereinafter.

To recapitulate, a cyclic peptide of formula I inhibits binding between the CS-1 peptide region of fibronectin and the VLA-4 receptor. Those inhibitors that are about five-times better inhibitors than the standard 10-mer peptide of SEQ ID NO:3 are contemplated here, each of the cyclic peptides of formulas I, above, and II and III, below, is at least about five times more potent (better) than the standard 10-mer.

In more preferred practice, a cyclic peptide inhibitor contains two to five residues between the two Xaa residues in that such cyclic peptides exhibit a greater potency in inhibiting binding between VLA-4-expressing cells and the standard 10-mer peptide of SEQ ID NO:3. The reason for the noted enhanced potency is unknown, but may be due to a reduction in the rotational and/or vibrational degrees of freedom present in these molecules with fewer residues between the Xaa residues that tends to restrict the inhibitor peptide in a conformation that is relatively more favorable for binding inhibition. These more preferred molecules typically exhibit binding inhibition

R^1-Cys-Phe-Leu-Asp-Phe-Y^3-Cys-NH_2 (SEQ ID NO:10-11)
III

wherein

the two Cys residues are oxidized to form a cystine residue;

R^1 is a C_1-C_4 acyl moiety or absent so that the peptide is terminated by the free α-amine of the oxidized Cys residue; and

Y^3 is Pro or Pro-Ser.

Thus, a still more preferred cyclic peptide inhibitor contains two oxidized Cys residues that form a cystine residue, as well as having a Z^1 Phe-Leu sequence between the N-terminal Cys and the Asp residue. These cyclic peptide inhibitors also include a Pro or Pro-Ser sequence as Y^3 between the Phe and C-terminal Cys residues. These still more preferred cyclic peptide inhibitors exhibit a relative potency that is about 100 to about 300 times greater than that exhibited by the standard 10-mer peptide of SEQ ID NO:3 in the in vitro assay discussed before.

The presently most preferred and most potent peptide inhibitor is a single peptide corresponding to formula IV

phenylacetyl-Cys-Asp-Phe-Cys-NH_2 IV
(SEQ ID NO:4)

wherein the two Cys residues are oxidized form a disulfide-containing cystine residue. This most preferred cyclic inhibitor peptide is about 500-600 times more potent in inhibiting binding between the CS-1 peptide and Jurkat cells than is the standard 10-mer of SEQ ID NO:3.

Exemplary cyclic inhibitor peptides are listed in Table 1 below using single letter

Table 1

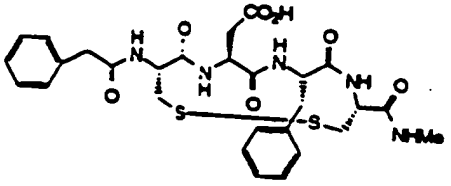
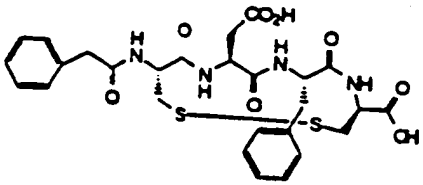
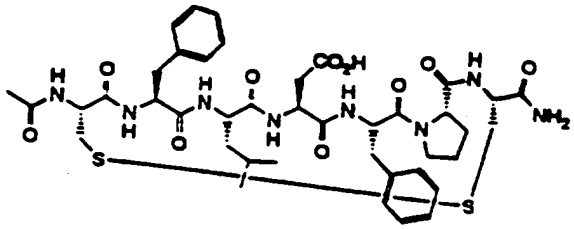
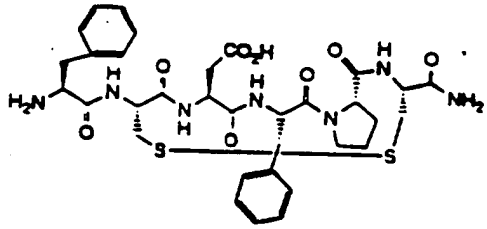
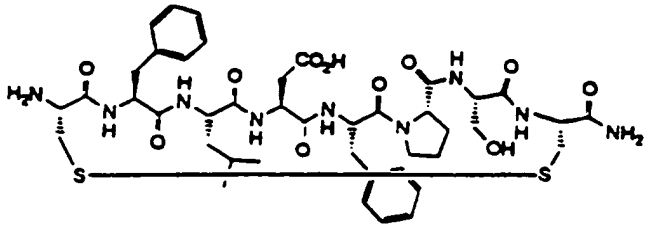
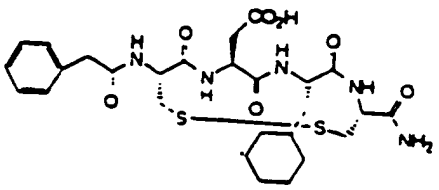
Relative Potencies of Cyclic Peptide Inhibitors*

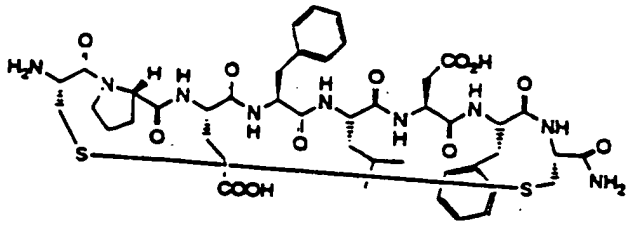
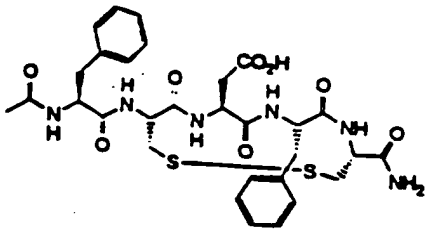
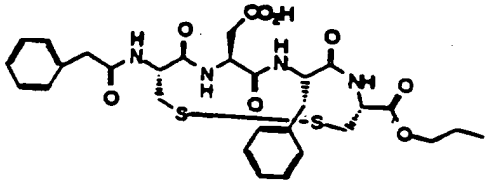
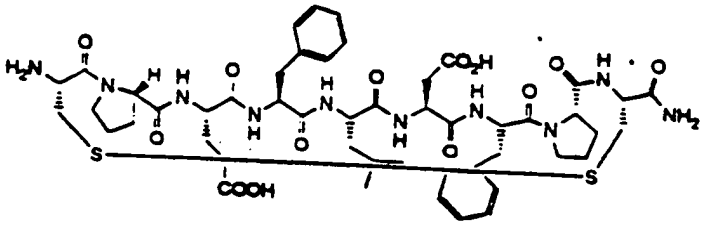
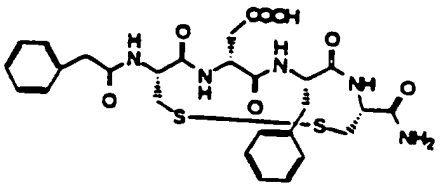
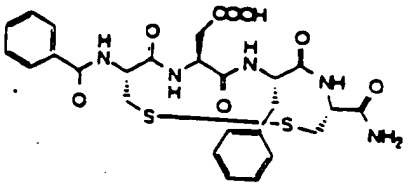
SEQ ID NO:	Sequence	Relative Potency
4	ΦAcCDFC-NH_2 [1]	954
10	CFLDFPC-NH_2	254
10	AcCFLDFPC-NH_2 [2]	135
32	FCDFPC-NH_2	131
11	CFLDFPSC-NH_2	122
--	ΦAcJDFC-NH_2 [1,3]	94
12	CFLDFPSTC-NH_2	81
34	AcFCDFC-NH_2 [2]	53
35	AcFCDFC-NH_2 [2,5]	41
33	CPEFLDFPC-NH_2	31
36	CPEFLDFPSC-NH_2	31
15	CEFLDFPC-NH_2	19
13	CFLDFPSTVC-NH_2	19
--	ΦAcCDF-$NHCH_2CH_2$- [7]	16
17	CEFLDFPSTC-NH_2	9
24	CGPEFLDFC-NH_2	9
16	CEFLDFPSC-NH_2	6
21	CPEFLDFPSC-NH_2	6
--	ΦAcJDFc-NH_2 [1,3,4]	4
37	FCDFC-NH_2	3
38	ILDVPILDVP-NH_2 [6]	2
39	ILDFP-NH_2 [6]	2
40	CLDFC-NH_2	1
41	AcFCDCP-NH_2 [2]	1
3	GPEILDVPST	1
42	FCDCP-NH_2	<1
--	AcLDV-NH_2 [6]	<1
--	AcLDF-NH_2 [6]	<1
43	SFDFS-NH_2 [6]	<1
--	LDV-NH_2 [6]	0
--	LDF-NH_2 [6]	0

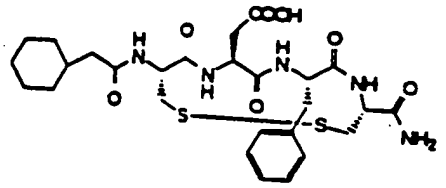
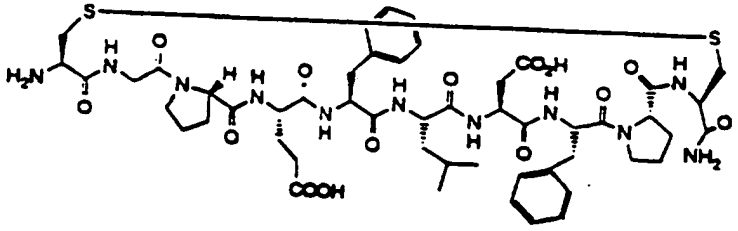
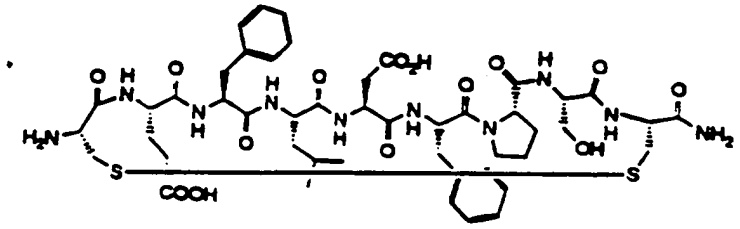
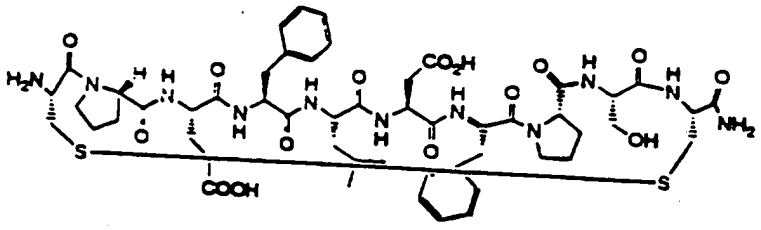
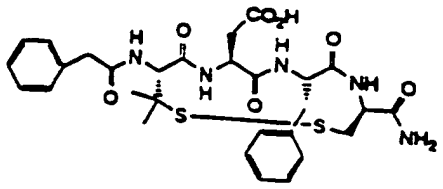
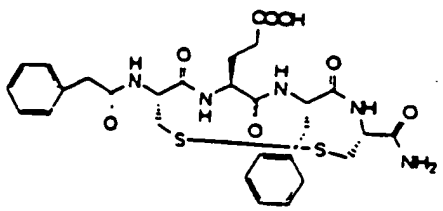
Table 1 still further illustrates the before-stated general preference for relativ ly shorter rather than longer sequences. Thus, all but one of the inhibitors that exhibited a relative potency of about 50 or greater contained a total of six or fewer residues. Contrarily, all but one of the peptide inhibitors that exhibited a relative potency of less than about 50 contained seven or more residues, or did not fulfill the requirements of a proviso, or did not contain a terminal carboxamide group.

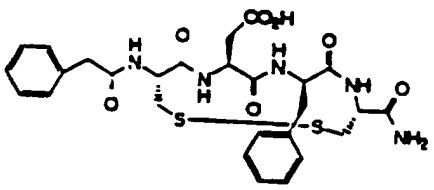
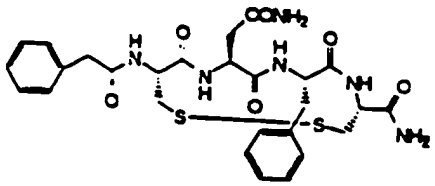
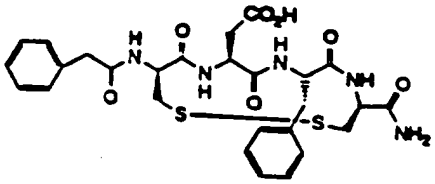
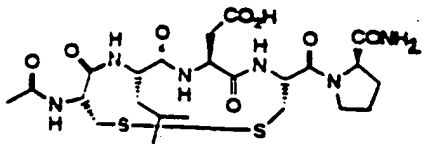
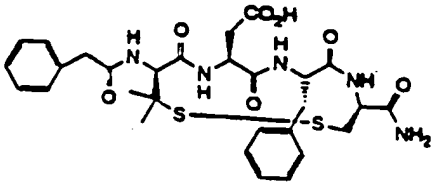
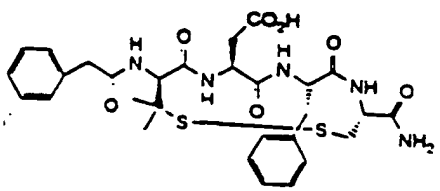
The data of Table 1 also illustrate the unexpected enhancements in binding inhibition exhibited by an inhibitor peptide contemplated herein as compared to other peptides of the art. For example, the Leu-Asp-Val (LDV) peptide stated as being a minimal peptide required for binding of the VLA-4 receptor in WO 93/12809 exhibited a relative inhibitory binding activity of about 1 as the N-acetyl C-amide derivative and zero as the N-free amine C-amide as compared to the peptide of SEQ ID NO:3. Similar results were observed with Leu-Asp-Phe (LDF) that is not disclosed in that published application, but can be present in a cyclic peptide here.

The chemical structure of exemplary cyclic inhibitor peptides are shown in Table 2 below. The in vitro binding inhibition potencies toward Jurkat cells relative to the standard peptide of SEQ ID NO:3 and the molecular ion mass spectroscopy data of such peptides are also shown in Table 2.

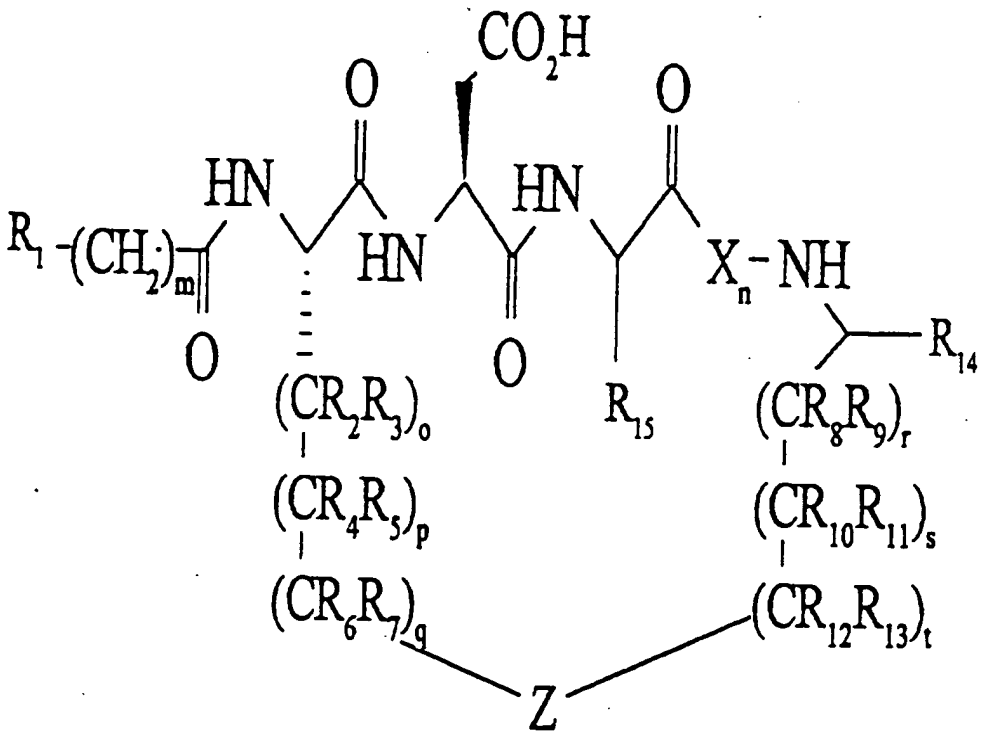
Structure	Molecular Ion	Relative Potency
	616 (MH^+)	232
	603 (MH^+)	202
		135
		131
		122
	608 (MH^+)	121

Structure	Molecular Ion	Relative Potency
		56.3
		52.6
	645 (MH$^+$)	46.6
		31.3
	602 (MH$^+$)	30.7
	588 (MH$^+$)	23.5

Structure	Molecular Ion	Relative Potency
	607 (MH+)	7.2
		6.26
		6.26
		6.26
		4.07
	616 (MH+)	3.13

Structure	Molecular Ion	Relative Potency
	602 (MH^+)	.63
	623 (MNa^+)	.63
		.59
		0
		0
		0

A preferred cyclic inhibitor peptide has the following formula:



In such a formula, Z is a linker group selected from the group consisting of -S-S-, -NH-C(O)-, -S-, and -C(O)-NH-. X_n is 0 to about 6 amino acids and subscript m is 0 to 5. R_1 is a phenyl, pyridyl or thiophenyl group optionally substituted by one or more lower alkyl, C_1 to C_3 alkoxy, halo, amino, C_1 to C_3 acylamino or nitro groups. The subscripts o, p, q, r, s, and t are, independently, 0 or 1 and R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, R_{11}, R_{12}, and R_{13} are, independently, a hydrogen atom or a lower alkyl or phenyl group. R_{14} is a hydrogen atom or a primary amide, carboxylic acid, lower alkyl ester, thiolo ester or mono or dihydroxy lower alkyl group. R_{15} is a phenyl, pyridyl, cyclohexyl, thiophenyl or primary

or a primary amid , carboxylic acid, lower alkyl ester, thiolo ester or mono or dihydroxy lower alkyl group. R_{15} is a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group or a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group wherein the lower alkyl group is a C_1 to C_3 alkyl straight or branch chain moiety or a salt or racemic mixture thereof.

The immediately above-cyclic inhibitory peptide wherein subscript m is 1, R_1 is a phenyl, pyridyl or thiophenyl group, subscript o and r are each 1, R_2, R_3, R_8, and R_9 are, independently, a hydrogen atom or a methyl group and R_{15} is a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group is also preferred.

group optionally substituted by a phenyl, pyridyl, cycl h xyl, thiophenyl or primary amide group wherein the lower alkyl group is a C_1 to C_5 alkyl straight or branch chain moiety or a salt or racemic mixture thereof.

The immediately above-cyclic inhibitory peptide wherein subscript m is 1, R_1 is a phenyl, pyridyl or thiophenyl group, subscript o and r are each 1, R_2, R_3, R_8, and R_9 are, independently, a hydrogen atom or a methyl group and R_{13} is a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group is also preferred.

each 1, R_2, R_3, R_8, and R_9 ar , ind pend ntly, a hydrogen atom or a methyl group and R_{15} is a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group is also preferred.

In addition to being more potent than the CS-1 or standard 10-mer peptides, a contemplated cyclic inhibitor peptide is relatively more stable in serum that is the CS-1 peptide. Thus, the cyclic inhibitor peptide at SEQ ID NO:4 exhibited no loss of potency after 24 hours in PBS at 7.2-7.4 that also contained 10 percent mouse or human serum. Contrarily, the CS-1 peptide lost its potency in less than one hour under the same conditions.

The straight chain polypeptides disclosed in jointly assigned, co-pending application Serial No. 08/164,101, filed December 6, 1993, were also assayed for their inhibition of CS-1 binding to VLA-4 and were found to be superior to CS-1 or the 10-mer standard used herein. When those peptides were assayed for the inhibition of binding of VLA-4 to recombinant, soluble VCAM-1 (sVCAM-1), their relative binding inhibition was about the same to worse when compared their CS-1 inhibitions, as is shown in Fig. 1.

Binding inhibition studies carried out using a herein contemplated cyclic inhibitor peptide such as previously discussed most preferred compound, showed an unexpected reversal of relative binding potencies. Thus, the best straight chain peptide of application Serial No. 08/164,101, having the sequence ϕAcLeuAspPhe-morpholinamide (XLDFZ) inhibited binding by CS-1 about 844-times more potently than did the Standard 10-mer of SEQ ID NO:3. Relative to th Standard 10-mer, the inhibition potencies of that XLDFZ peptide, the XLDFp and the most preferred cyclic peptide of SEQ ID NO:4 were

Standard solid phase synthesis was used here. Thus, the N-protected, C-terminal residue was linked to a solid support having a benzhydrylamine or benzhydryl alcohol substituent. Fmoc-amine blocking groups were used in these syntheses, although t-Boc, CBZ or other blocking groups can also be used with other solid supports. Upon deblocking the Fmoc group with piperidine, another residue was coupled. That coupling was followed by further deblocking, coupling, deblocking etc. steps until a solid phase-linked peptide of desired sequence was prepared. As appropriate to each peptide, an N-terminal R group was added after a final N-deblocking step. The desired peptide and some accompanying functional group protecting groups were removed from the solid support by reaction with trifluoroacetic acid (TFA). This procedure results in a C-amide-terminated peptide when a benzhydrylamine solid support is used. This procedure can be performed prior to or following the cyclization reaction described below.

Contemplated peptides can also be prepared using t-Boc N-protecting groups and another solid support, or a benzylamino-substituted solid support to which a p-hydroxymethylphenylcarboxyl (PAM) group is first reacted with the amine of the support to form a carboxamide. The hydroxyl group is then used to form an ester link to the first peptide and standard t-Boc synthetic technology is thereafter followed. Reaction of the completed, deprotected solid phase-linked peptide with ammonia provides the C-terminal amide peptide.

In other embodiments, liquid phase peptide syntheses can be utilized. For example, a C-amido N-free amino group-containing amino acid derivative is coupled in solution to the carboxyl of a t-Boc-protected residue using a carbodiimide. The t-Boc protecting group is removed from the resulting

dimethylformamide. Following standard work-up and purification, the bezyl ester sid chain protecting group of the aspartic acid residue was removed by hydrogenolysis to give the desired cyclic sulfide [Y.-B. He, Z. Huang, K. Raynor, T. Reisine and M. Goodman, supra].

Regardless of the synthetic method used, an inhibitor peptide is typically recovered and purified prior to use. Recovery and purification techniques are well known and will not be dealt with here.

Salt forms of the cyclic inhibitor peptides can also be made using well known methods. A preferred salt is a pharmaceutically-acceptable salt. Such salts include salts formed with the organic and inorganic cations such as those chosen from the alkali and alkaline earth metals, for example, lithium, sodium, potassium, barium and calcium; ammonium; and the organic cations, for example, dibenzlammonium, benzylammonium, 2-hydroxyethylammonium, bis(2-hydroxyethyl)ammonium, phenylethylbenzylammonium, and dibenzylethylenediammonium). Other cations encompassed include the protonated form of procaine, quinine, N-methylglucosamine and basic amino acids. A preferred cation for the carboxylate anion is sodium cation.

C. Mass Spectroscopy

Mass spectroscopy data confirmed the expected molecular weight of exemplary cyclic inhibitor peptides. The data was obtained using fast atom bombardment mass spectrometry (FAB), electrospray mass spectrometry (Electrospray), or matrix assisted laser desorption mass spectroscopy (MALDI-TOF-MS).

Bri fly, FAB was done using a VG ZAB-VSE double focusing high resolution mass spectrometer equipped with a cesium ion gun. The mass

was the Alpha cyano-4-hydroxy-cinnamic acid, gentisic acid or sinapinic acid. The laser was an N2 laser.

The mass spectroscopy data of exemplary cyclic inhibitor peptides is shown in Table 2.

D. Prodrug cyclic peptides

Prodrug cyclic peptides are transformed in vivo from compounds that do not necessarily bind the VLA-4 receptor in vitro to compounds having such binding activity in vivo. Chemical modifications of drugs that make prodrugs are known in the art and include, for example, esters of carboxylic acids or carboxyamide phosphonate groups. Moreover, the synthesis of prodrugs is by well known methods and will not be detailed here. See, for example, Bundraard, Design of Prodrugs, Elsevier Science Pub. Co., N.Y. (1985), and Prodrugs as Novel Drug Delivery Systems Symposium, 168th Annual Meeting, American Chemical Society, Atlantic City, N.J., Eds. T. Higuchi and V. Stella, ACS Symposium Serries 14, 1975, which are herein incorporated by reference.

A prodrug cyclic peptide also can be a cyclic peptide ester that increases blood levels, prolongs the efficacy or makes orally available the corresponding non-esterified form. Such ester groups include lower alkoxymethyl groups, for example, methoxymethyl, ethoxymethyl and iso-propoxymethyl. Other such ester groups include α-(C_1 to C_4) alkoxyethyl groups, for example, methyoxylethyl, ethoxyethyl, propoxyethyl and isopropoxyethyl; alkylthiomethyl groups, for example, methylthiomethyl, and ethylthiomethyl; 2-oxo-1,3-dioxolen-4-methyl groups, for example, 2-oxo-1,3-dioxolen-4-ylmethyl; acyloxymethyl groups, for exampl , pivaloyloxymethyl; ethoxycarbonyl-1-methyl group; α-acyloxy-α-substituded methyl groups; and (C_1 to C_4 alkyloxycarbonyloxy)ethyl groups.

E. Compositions and Processes

results. That inhibition of emigration of inflammatory c lls results in a reduction of the fibronectin CS-1/VLA-4-mediated inflammatory response caused by those inflammatory cells, and thereby reduces the observed inflammation.

Particular inflammatory disease states that are mediated by CS-1 and VLA-4, and in which a contemplated inhibitor peptide can diminish inflammation are quite broad. Illustrative of those types of inflammation are asthma, arthritic conditions such as rheumatoid arthritis and osteoarthritis, allograft rejection, various types of skin inflammation, and demyelinating diseases of the central nervous system.

Specific pathological inflammatory conditions in which expression of CS-1 has been found to be implicated and where no such expression is observed in absence of a pathological condition (i.e., in normal tissue) include: rheumatoid arthritis (synovium), osteoarthritis (synovium), skin psoriasis, kidney transplant, asthmatic lung, and lymph node high endothelial venules (HEV) in humans, as well as in the gut of monkeys infected with SIV and those having inflammatory bowel disease, rabbits having asthmatic lungs and heart transplants, mouse brain in experimental autoimmune encephalomyelitis (EAE) and skin in delayed type hypersensitivity (DTH), and the joints of rats with induced arthritis.

A pharmaceutical composition containing a before-discussed cyclic inhibitor peptide such as a peptide of formula I dissolved or dispersed in a pharmaceutically acceptable carrier or diluent that is preferably aqueous is also contemplated for use in treating a CS-1/VLA-4-m diated inflammatory disease state such as those discussed befor . Such a composition contains a CS-1/VLA-4 binding-inhibiting

intramuscularly or interperitoneally. Thus, a composition for intravenous administration is particularly contemplated that comprises a solution of a contemplated inhibitor peptide dissolved or dispersed in a pharmaceutically acceptable diluent (carrier), preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.9 percent saline, buffered aqueous ethanol solutions and the like. These compositions can be sterilized by conventional, well known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. A composition can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of cyclic inhibitor peptide utilized is usually at or at least about 0.0001 percent to as much as about 0.1 percent by weight and is selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intravenous infusion can be made up to contain 250 ml of sterile Ringer's solution normal saline or PBS, and up to about 2.5 mg of a cyclic inhibitor peptide. Actual methods for preparing parenterally administrable compounds are known or apparent to those skilled in the art and are described in more detail in for example, Remington's Pharmaceutical

esters, such as mixed or natural glycerides can be employed. The surfactant can constitute about 0.1 to about 20 percent by weight of the composition, and preferably about 0.25 to about 5 percent. The balance of the composition is ordinarily propellant. Liquefied propellants are typically gases at ambient conditions, and are condensed under pressure. Among suitable liquefied propellants are the lower alkanes containing up to 5 carbons, such as butane and propane; and preferably fluorinated or fluorochlorinated alkanes. Mixtures of the above can also be employed. In producing the aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve. A pump-activated spray using air as propellant (atomizer or nebulizer) is also contemplated.

For example, for the treatment of asthma in rabbits, the dose of a contemplated cyclic peptide is in the range of about 1 to 100 mg/day for a 2-3 kg animal. For a human asthma patient, that dose is in the range of about 1 to about 100 mg/day for a 70 kg patient. Administration for asthma is typically by aerosol from a nebulizer. Ideally, therapeutic administration should begin as soon as possible after the attack begins.

A pharmaceutical composition containing a cyclic inhibitor peptide can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, a composition is administered to a patient already suffering from a disease, as described above, in an amount sufficient to inhibit binding between VLA-4-expressing leukocytes and endothelial cells that express the CS-1 peptide portion; i.e., r duce inflammation and

(five-times more potent), when used at on -fifth the molar amount of the 10-mer standard is a useful binding-inhibiting amount. More preferably, the amount is about one-fiftieth the amount of the 10-mer. More preferably still, the amount is equal to about one-hundredth that of the 10-mer. Inasmuch as those amounts inhibit binding by about 50 percent, greater concentrations that inhibit binding still further are preferred.

Thus, for in vitro use, a minimal CS-1/VLA-4-inhibiting amount is the IC_{50} value. For in vivo use, the CS-1/VLA-4-inhibiting amount usually used begins with the IC_{50} value concentration, and can decrease as required or one can increase to the solubility limit of the cyclic peptide in the utilized aqueous medium; i.e., the aqueous medium at pH 7.2-7.4 used such as normal saline where parenteral administration is used or intestinal fluid where oral administration is used.

Single or multiple administrations of a composition can be carried out with dose levels and pattern being selected by the treating physician or veterinarian. In any event, a pharmaceutical composition is formulated to provide a quantity of a cyclic inhibitor peptide sufficient to effectively treat the patient.

A process for treating fibronectin CS-1/VLA-4-mediated inflammation is also contemplated. In accordance with such a process, a before-described, contemplated cyclic inhibitor peptide is administered to a mammal in need of such a treatment such as a mammal having such inflammation or prophylactically as prior to an allograft. This administration is preferably via a before-discussed pharmaceutical composition. The cyclic peptide is administered in an inflammation-reducing (CS-1/VLA-4-inhibiting) amount. The mammal such as mouse, rat,

The standard deprotection/coupling cycl iterated during this synthesis is described in terms of the first coupling of Fmoc-Cys(Trt) to the Rink amide MBHA resin: The loading of the starting resin was 0.5-0.7 mmol/g polystyrene, and 0.1 or 0.25 meq were used in each synthesis. A typical reaction cycle proceeded as follows: (1) the N-terminal Fmoc group was removed with 25 percent piperidine in dimethylformamide (DMF) for 5 minutes, followed by another treatment with 25 percent piperidine in DMF for 15 minutes. The resin was washed five times with DMF. An N-methylpyrolidone (NMP) solution of a 4 to 10 fold excess of a pre-formed 1-hydroxybenzotriazole ester of the appropriate Fmoc-amino acid [Fmoc-Cys(Trt)] was added to the resin and the mixture was allowed to react for 30-90 minutes. The resin was washed with DMF in preparation for the next elongation cycle.

Example 2: <u>In Vitro Binding Assays</u>

Jurkat cells (ATCC TIB 152), a human T lymphoblastic line, labeled with ^{51}chromium were used to assay <u>in vitro</u> binding inhibition provided by various peptides discussed herein. Costar™ 96 well flat-bottom microtiter plates (catalog No. 9050, Cambridge, MA) were found to provide the best results in these assays.

The plates were prepared as follows: The 25-mer CS-1 peptide (SEQ ID NO:1) dissolved at 0.5-1 μg/ml in a buffer of 0.1M $NaHCO_3$ at pH 9.5 that also contained 10 μg/ml of bovine serum albumin (BSA) or a conjugate of the CS-1 peptide linked to ovalbumin (CS-1-OVA) dissolved at 1-2.5 μg/ml in the same buffer was used as the substrate. Each well of the microtiter plates was coated with 50 μl of substrate or buffer alone for controls. The wells were permitted to dry out completely and w re then rinsed

typical initial concentrations were 10 μg/ml, 2 μg/ml, 0.4 μg/ml and 0.08 μg/ml.

The ^{51}Cr-labeled cells ($1X10^6$ cells at 60 μl/well) were then admixed with the diluted cyclic peptide solutions. The admixtures were maintained at room temperature (about 22°C) for 30 minutes.

One hundred microliters of each inhibitor peptide/cell admixture were transferred to the substrate-coated wells. This was done in triplicate for each dilution. The resulting plates were incubated for 30 minutes at 37°C and then washed gently three times with RPMI/1 percent BSA at 200 μl/well. Binding was observed microscopically, particularly after the second wash.

The bound cells were then lysed by the addition of a 0.5 percent solution of sodium dodecylsulfate in water at 100 μl/well. The resulting solutions were then processed for counting and calculation of IC_{50} values following usual procedures. Appropriate positive and negative controls were used with each plate so that the results of separate assays could be normalized and compared.

The potency data of Table 1 and Table 2 are so normalized. The absolute IC_{50} value for the peptide of SEQ ID NO:4 is 0.3 μM.

Assays conducted using sVCAM-1 as solid phase-bound antigen were carried out substantially identically to those using CS-1. The sVCAM-1 polypeptide used had the same amino acid residue sequence as the materials described in Lobb et al., Biochem. Biophys. Res. Commun., 178:1498-1504 (1991) and Cybulsky et al., Proc. Natl. Acad. Sci., USA, 88:7859-7863 (1991). Human embryonal kidney line 293 (ATCC CRL 1573) cells were co-transfected with a plasmid (pCDNAI;invitrogen) containing th sVCAM-1 DNA sequence and plasmid pSV2-neo [Southern et al.,

challenge or salin injection is measured with a microcaliper 24 hours th reafter.

The results of this study show that administration of a contemplated inhibitor peptide reduces this type of CS-1/VLA-4-mediated immunoinflammation as compared to the untreated controls. Use of the control peptide provides no reduction of inflammation.

Example 4: Treatment of Asthmatic Rabbits

Six New Zealand white rabbits are immunized with house dust mite antigen from birth through four months of age. Upon immunization, three rabbits receive a single nebulizer administration of a cyclic inhibitor peptide such as the cyclic peptide of SEQ ID NO:4 in aqueous 50 percent ethanol as diluent in an amount of 100 mg/kg, and the other three receive diluent alone. All of the rabbits are challenged with house dust mite antigen about 15-30 minutes after administration of the peptide, with those animals not receiving cyclic inhibitor peptide serving as controls.

Once immunized and challenged, the inflammatory state subsides to a basal level within about three weeks. The three animals used as controls are thereafter used as subjects for receipt of an inhibitor peptide, and the three rabbits that initially received the peptide serve as controls.

Using such a crossover study, the three initial control rabbits are treated with the above cyclic inhibitor peptide in the above diluent at a time more than three weeks after the above study, and the three previous recipients of the cyclic peptide are administered th diluent alone. All six are then challenged again.

Initial pulmonary function, measured by dynamic compliance (C_{dyn}) and lung resistance (R_L), and

H re, seven rabbits are injected on day zero with 1 mg/kg/day of the inhibitor peptide such as the cyclic peptide of SEQ ID NO:4 in aqueous diluent. The two rabbits then each receive a grafted heart, with the graft being made in the carotid artery to the aorta of the grafted heart, and the jugular vein to the pulmonary artery of the grafted heart. The rabbits thereafter receive daily injections of the same dose of that peptide, and are sacrificed on day 7. Another seven animals receive normal saline injections in place of the peptide injections, also receive a similarly allografted heart, and are similarly sacrificed at day 7.

The animals' blood vessels are thereafter examined histologically for evidence of arteriopathy, and particularly thickening of the intimal elastic lamina (IEL) layer of the coronary arteries. Thickening of the IEL is caused at least in part by emigration of effector cells bearing the CD2 marker such as T cells and NK cells. Clausell et al., Am. J. Path., 142(6):1772-1786 (1993).

These rabbits are maintained on a high fat diet to help accelerate the effects of rejection. As a result of the diet, basal levels of IEL thickening and lesion severity are elevated relative to rabbits fed on a normal diet. Basal levels are assayed in the coronary arteries of the hearts of recipient animals, whereas IEL thickening and lesion severity are assayed in the coronary arteries of the grafted hearts.

Upon examination of vessels of the saline-treated rabbits, a large percentage of the vessels exhibit IEL thickening. Similar evaluation of vessels from the rabbits that receiv the inhibitor peptide treatment show that a smaller percentage of the vessels exhibit IEL thickening.

PLP emulsified in a 1:1 mixture of PBS and complete Freund's adjuvant (CFA). Each mouse is injected with 0.2 ml of the adjuvant emulsion subcutaneously (s.c.) at two sites in the hind flank. All mice receive 10^7 killed Bordetella pertussis units in 100 µl and are injected intravenously 24 to 72 hours later.

Mice are observed daily, beginning at day 8 for clinical signs of EAE, and disease is scored on a scale of 0-5 as: 0 = no disease; 1 = floppy tail; 2 = moderate hind limb weakness; 3 = paraparesis; 4 = paraplegis with moderate forelimb weakness; 5 = quadriplegis or premoribund state.

A cyclic inhibitor peptide such as that of SEQ ID NO:32 is administered intraperitoneally at 1 mg/mouse in 0.2 ml of incomplete Freund's adjuvant at days 8 and 9. A control peptide is similarly administered.

Summed or averaged scores for clinical signs are plotted vs. time. The area under the resulting curves is calculated between day 8 and day 35 to calculate percentage inhibition of EAE by a cyclic inhibitor peptide. The percent inhibition is calculated as follows:

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Area of cyclic inhibitor peptide + control area}}{100}\right) X$$

Animals treated with a cyclic inhibitor peptide contemplated herein exhibit marked improvement in clinical signs as compared to those animals treated with the control peptide.

Example 8: CS-1 Expression in Human Rheumatoid Arthritis

Surgically-obtained synovial specimens from human rheumatoid arthritis (RA) pati nts are examined microscopically for the expression of the CS-1

Although the present invention has now been described in terms of certain preferred embodiments, and exemplified with respect thereto, one skilled in the art will readily appreciate that various modifications, changes, omissions and substitutions may be made without departing from the spirit thereof.

20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^2 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa

(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^3 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note="Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the sixth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Asp Phe Pro Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^3 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7
(D) OTHER INFORMATION: /note="Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the seventh position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Xaa Asp Phe Pro Ser Thr Xaa
1 5

(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "Both Xaa groups ar α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the sixth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Xaa Phe Leu Asp Phe Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^2 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the seventh position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Xaa Phe Leu Asp Phe Pro Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Xaa Phe Leu Asp Phe Pro Ser Thr Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^2 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the tenth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Phe Leu Asp Phe Pro Ser Thr Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^2 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl,

(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note="Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the ninth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Xaa Glu Phe Leu Asp Phe Pro Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note="Both Xaa gr ups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the tenth position has a C-terminal

benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..8
(D) OTHER INFORMATION: /note="Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the eighth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Pro Glu Phe Leu Asp Phe Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^1 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note="Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the ninth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Xaa Pro Glu Phe Leu Asp Phe Pro Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Xaa Pro Glu Phe Leu Asp Phe Pro Ser Thr Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /note="Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the twelfth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Xaa Pro Glu Phe Leu Asp Phe Pro Ser Thr Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptid

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..11
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..11
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the eleventh position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Xaa Gly Pro Glu Phe Leu Asp Phe Pro Ser Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or

1

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Pro Glu Phe Leu
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Pro Ser Thr Val
1

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "The carboxy-terminus is a carboxamide."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Phe Cys Pro Phe Pro Cys
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note= "The carboxy-terminus is a carboxamide."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note="The amino-terminus has a acetyl group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Phe Cys Asp Phe Cys
1 5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the ninth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Xaa Pro Glu Leu Asp Phe Pro Ser Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note= "The carboxy-terminus is a carboxamide."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Cys Leu Asp Phe Cys
1 5

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note= "The carboxy-terminus is a carboxamide."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note= "The amino-terminus has a acetyl group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Phe Cys Asp Cys Pro
1 5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..4

carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Xaa Pro Glu Leu Asp Phe Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tryosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl."

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "Both Xaa groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which the Xaa in the tenth position has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Xaa Pro Glu Leu Asp Phe Pro Ser Thr Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..11
(D) OTHER INFORMATION: /note= "The amino-terminus has an R group where R is (a) R^1 and R^1 is either (i) absent or (ii) a C_1-C_4 acyl moiety or (b) R^2 and R^2 is

CLAIMS

1. A cyclic peptid of the formula

$R\text{-}Xaa^1\text{-}Z\text{-}Asp\text{-}Phe\text{-}Y\text{-}Xaa^2$ (SEQ ID NO:4-28)

wherein

R is

(a) R^1 that is (i) absent so that the peptide is terminated by the free α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or

(b) R^2 that is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tyrosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl;

the Xaa^1 and Xaa^2 groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which Xaa^2 has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen;

Z is absent, or a peptide selected from the group consisting of Pro-Glu-Leu, Phe-Leu, Glu-Phe-Leu, Pro-Glu-Phe-Leu (SEQ ID NO:29), and Gly-Pro-Glu-Phe-Leu (SEQ ID NO:30); and

Y is absent, Pro, or Y^1 that is a peptide selected from the group consisting of Pro-Ser, Pro-Ser-Thr and Pro-Ser-Thr-Val (SEQ ID NO:31);

with the provisos that:

(i) R is R^2 when Z is absent, and

(ii) R^2 is selected from the group consisting of phenylacetyl, benzoyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl when the two Xaa residues are separated by two amino acid residues.

2. The cyclic p ptide according to claim 1 wherein Xaa^1 and Xaa^2 together form a sulfide bond in a chain that contains 3 to about 6 atoms.

5. The cyclic peptide according to claim 4 wherein the ring structure of said Z is absent.

6. The cyclic peptide according to claim 5 wherein said Y is absent.

7. The cyclic peptide according to claim 4 wherein said Y is absent.

8. The cyclic peptide according to claim 4 wherein R is R^1.

9. A cyclic peptide of the formula
$R\text{-}Xaa\text{-}Z^1\text{-}Asp\text{-}Phe\text{-}Y^2\text{-}Xaa\text{-}NH_2$ (SEQ ID NO:5-7, 9-12)
wherein
R is
(a) R^1 that is (i) absent so that the peptide is terminated by the free α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or
(b) R^2 that is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tyrosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl;
at least one Xaa is an oxidized cysteine and the other Xaa is an oxidized cysteine, homocysteine or penicillamine residue such that the two Xaa's together form a disulfide bond;
Z^1 is absent or Phe-Leu; and
Y^2 is absent, Pro, Pro-Ser or Pro-Ser-Thr;
with the provisos that:
(i) R is R^2 when Z^1 is absent, and
(ii) R^2 is selected from the group consisting of phenylacetyl, benzoyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl when the two Xaa residues are separated by two amino acid residues.

(a) R^1 that is (i) absent so that the peptide is terminated by the fre α-amine of Xaa or (ii) a C_1-C_4 acyl moiety, or

(b) R^2 that is selected from the group consisting of phenylacetyl, benzoyl, phenylalanyl, tyrosyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl;

the Xaa^1 and Xaa^2 groups are α-amino acid residues that together form a sulfide or disulfide bond in a chain that contains 3 to about 6 atoms, including at least one sulfur atom between the α-carbons of the two Xaa groups, and in which Xaa^2 has a C-terminal carboxamide group, or the C-terminal carboxyl is replaced by hydrogen;

Z is absent, or a peptide selected from the group consisting of Pro-Glu-Leu, Phe-Leu, Glu-Phe-Leu, Pro-Glu-Phe-Leu (SEQ ID NO:29), and Gly-Pro-Glu-Phe-Leu (SEQ ID NO:30); and

Y is absent, Pro, or Y^1 that is a peptide selected from the group consisting of Pro-Ser, Pro-Ser-Thr and Pro-Ser-Thr-Val (SEQ ID NO:31);

with the provisos that:

(i) R is R^2 when Z is absent, and

(ii) R^2 is selected from the group consisting of phenylacetyl, benzoyl, N-C_1-C_4 acyl phenylalanyl and N-C_1-C_4 acyl tyrosyl when the two Xaa residues are separated by two amino acid residues.

17. The process according to claim 16 wherein said administration is by inhalation.

18. The process according to claim 16 wherein said administration is parenteral.

20. The pharmaceutical composition according to claim 19 wherein Z is absent.

21. The pharmaceutical composition according to claim 19 wherein Y is absent.

22. The pharmaceutical composition according to claim 19 wherein Z is Phe-Leu and Y is Pro.

23. The pharmaceutical composition according to claim 19 wherein said cyclic peptide is selected from the group consisting of SEQ ID NO'S:4, 10, 32 and 11, and N-phenylacetyl-penicillaminyl-Asp-Phe-Cys-NH_2.

R_{14} is a hydrogen atom or a primary amide, carboxylic acid, lower alkyl ester, thiolo ester or m no or dihydroxy lower alkyl group; and

R_{15} is a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group or a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group;

wherein the lower alkyl group is a C_1 to C_3 alkyl straight or branch chain moiety or a salt thereof.

25. A cyclic inhibitor peptide of claim 24 wherein o and r are each 1, p, q, s, and t are 0 and R_2, R_3, R_8 and R_9 are, independently, a hydrogen atom or a lower alkyl group.

26. A cyclic inhibitor peptide of claim 24 wherein X_n is 0.

to C_5 alkyl straight or branch chain moiety or a salt thereof.

28. The cyclic inhibitor peptide of claim 27 wherein m is 1.

29. The cyclic inhibitor peptide of claim 27 wherein R_1 is a phenyl, pyridyl or thiophenyl group.

30. The cyclic inhibitor peptide of claim 27 wherein o and r are each 1 and R_2, R_3, R_8, and R_9 are, independently, a hydrogen atom or a methyl group.

31. The cyclic inhibitor peptide of claim 30 wherein R_{15} is a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group.

32. The cyclic inhibitor peptide of claim 27 wherein m is 1, R_1 is a phenyl group, R_2, R_3, R_8, and R_9 are each a hydrogen atom, R_{14} is a methyl ester group and R_{15} is a N-phenylacetyl group.

R_{14} is a hydrogen atom or a primary amide, carboxylic acid, lower alkyl ester, thiolo ester or mono or dihydroxy lower alkyl group; and

R_{15} is a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group or a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group;

wherein the lower alkyl group is a C_1 to C_5 alkyl straight or branch chain moiety or a salt thereof.

34. The cyclic inhibitor peptide of claim 33 wherein m is 1.

35. The cyclic inhibitor peptide of claim 33 wherein R_1 is a phenyl, pyridyl or thiophenyl group.

36. The cyclic inhibitor peptide of claim 33 wherein o and r are each 1 and R_2, R_3, R_8, and R_9 are, independently, a hydrogen atom or a methyl group.

37. The cyclic inhibitor peptide of claim 36 wherein R_{15} is a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group.

38. The cyclic inhibitor peptide of claim 33 wherein m is 1, R_1 is a phenyl group, R_2, R_3, R_8, and R_9 are each a hydrogen atom, R_{14} is a primary amine group and R_{15} is a N-phenylacetyl group.

to C_3 alkyl straight or branch chain moiety or a salt thereof.

40. The cyclic inhibitor peptide of claim 39 wherein m is 1.

41. The cyclic inhibitor peptide of claim 39 wherein R_1 is a phenyl, pyridyl or thiophenyl group.

42. The cyclic inhibitor peptide of claim 39 wherein o, r and s are each 1 and R_2, R_3, R_8, and R_9 are, independently, a hydrogen atom or a methyl group.

43. The cyclic inhibitor peptide of claim 39 wherein R_{15} is a lower alkyl group optionally substituted by a phenyl, pyridyl, cyclohexyl, thiophenyl or primary amide group.

44. The cyclic inhibitor peptide of claim 42 wherein m is 1, R_1 is a phenyl group, R_2, R_3, R_8, and R_9 are each a hydrogen atom, R_{14} is a hydrogen atom and R_{15} is a N-phenylacetyl group.

45. A prodrug cyclic peptide comprising the ester of the cyclic inhibitor peptide of claim 24, 27, 33 or 39.

46. A pharmaceutical composition comprising the cyclic inhibitor salt of claim 24, 27, 33 or 39 and a pharmaceutically acceptable carrier.

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(57) Abstract

The present invention contemplates a cyclic peptide that inhibits the binding between the VLA-4 receptor expressed on inflammatory leukocytes and the fibronectin CS-1 peptide expressed on endothelial cells that are involved in immunoinflammatory disease states. Pharmaceutical compositions containing a contemplated cyclic peptide and processes for treating immunoinflammatory conditions using a binding-inhibitory cyclic peptide are also disclosed.

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A. CLASSIFICATION OF SUBJECT MA[illegible]ER
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According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 15958 (TANABE SEIYAKU CO) 21 July 1994 see claims 1,4	1-46
Y	see page 7, line 8-14 see page 9, paragraph 1 see page 59 see table 3	1-46
Y	WO,A,92 00995 (TANABE SEIYAKU CO) 23 January 1992 *COMPOUNDS NO. 100,102,104,121,122 see page 87-88 *See definition of X1* see page 89; claim 1	1-46

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Deffner, C-A

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9415958	21-07-94	CA-A-	2153228	21-07-94
		EP-A-	0677060	18-10-95
WO-A-9200995	23-01-92	US-A-	5192746	09-03-93
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